# Sortase

### Week 8

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

## Summary

# 1 Digestion of pSB1C3 and Gb1-SortA PCR Product

## Responsible

Reskandi Rudjito and Betty Tesfai

## Protocols used

- 3A Assembly (Digestion and Ligation)

## Modifications and comments to protocols

The amount of enzyme used was adjusted to the concentration of the DNA sample.

# Experimental Set Up

The experimental set up for the digestions are stated in the tables below. A reaction volume of 10 $\mu$l was used for both of the digestions.

Table 1: Concentrations of DNA to be digested

| Sample | Concentration [ng/$\mu$l ] |
|---|---|
| pSB1C3 | 41.0 |
| Gb1-SortaA PCR product 1 | 383.0 |

Table 2: Digestion reaction of pSB1C3

| Reaction | Volume [$\mu$l ] |
|---|---|
| DNA | 5 |
| Buffer SH 10 X | 1.0 |
| Pst1 | 0.2 |
| EcoR1 | 0.2 |
| Sterile water | 3.6 |

The digestion of pSB1C3 was done in duplicates.

Table 3: Digestion reaction of Gb1-SortA PCR Product

| Reaction | Volume [$\mu$l ] |
|---|---|
| DNA | 1.5 |
| Buffer SH 10 X | 1.0 |
| Pst1 | 0.5 |
| EcoR1 | 0.5 |
| Sterile water | 6.5 |

# Sample Calculation

1 $\mu$l of restriction enzyme is used for every 1 $\mu$g/$\mu$l of DNA.

## Results and Conclusions

The digested pSB1C3 and Gb1-SortA was subjected to gel electrophoresis for analysis. The sample for digested Gb1-SortA had difficulties in loading and therefore it is not shown on the gel below. The DNA bands of the digested pSB1C3 shows the expected size of around 2200 bp.

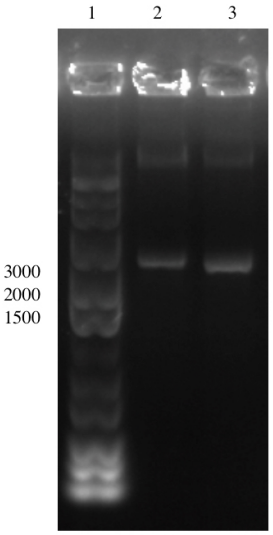


Figure 1: Digested pSB1C3. (1) Ladder (2) Digested pSB1C3 (3) Digested pSB1C3

Table 4: Concentration of digested products after purification

| Sample | Concentration [ng/$\mu$l ] |
|---|---|
| Digested pSB1C3 (1) | 11.5 |
| Digested pSB1C3 (2) | 8.9 |
| Digested Gb1-SortA | 6.9 |

# 2 Ligation of and Transformation of pSB1C3-GB1-SortA

## Responsible

Aman Mebrahtu

## Protocols used

- 3A Assembly (Digestion and Ligation)
- Transformation

## Modifications and comments to protocols

Only two fragments were used in the ligation procedure.

# Experimental Set Up

In order to perform the ligation, the two DNA fragments consisting of the Gb1-SortA and pSB1C3 backbone are digested and purified prior to ligation. The concentrations of each DNA fragment are stated in Table 5.

Table 5: Concentrations DNA fragments for Ligation

| Sample | Concentration [ng/$\mu$l] | Size [bp] |
|---|---|---|
| Digested Gb-SortA | 6.9 | 669 |
| Digested pSB1C3 | 8.9 | 2200 |

The ligation was performed overnight at a temperature of 16°C and was afterwards transformed into both TOP 10 and BL21 cells.

Table 6: Re-Transformation of pSB1C3-T7-Lys

| Sample | Comment |
|---|---|
| pSB1C3-GB1-SortA Top10 | Transformed into Top10 cells |
| pSB1C3-GB1-SortA BL21 (DE3) | Transformed into BL21 (DE3) cells |
| positive control Top 10 | No antibiotics |
| positive control BL21 | No antibiotics |

# Sample Calculation

In this ligation, we have decided to go forward with an insert:vector molar ratio of 10:1. The calculations shown on the tables below are adjusted so that each reaction contains 50 ng of backbone and the inserts are adjusted according to the desired molar ratio. Since the concentrations of the purified DNA fragments were relatively low, this resulted in an overload of reaction volume which is indicated by a minus sign for sterile water.

Table 7: Ligation Reaction of pSB1C3-Gb1-SortA

| Component | Volume |
|---|---|
| Gb1-SortA | 11.02 |
| pSB1C3 | 2.81 |
| T4 DNA Ligase | 0.5 |
| T4 DNA Ligase Buffer 10X | 1.5 |
| Sterile water | -0.83 |

## Results and Conclusions

Two colonies were observed from pSB1C3-Gb1-SortA transformed in BL21 cells. The colonies were subjected to further analysis by colony picking and sequencing.

# 3 Colony picking and Colony PCR of Transformed colonies containing pSB1C3-Gb1-SortA

## Responsible

Reskandi Rudjito

## Protocols used

- Colony picking
- Gel electrophoresis

# Experimental Set Up

The two colonies that were successfully transformed with pSB1C3-Gb1-SortA was picked and grown on LB + antibiotics. To confirm the correct insert was cloned into the vector, we performed a colony PCR with the following conditions.
The PCR was done with verification primers (VF2 and VR). Table 8 shows the composition used for the PCR and Table 9 shows the PCR conditions used in the thermocycler. The PCR reaction used for each sample was 25 $\mu$l.

Table 8: PCR Reaction

| Master Mix | Component | Volume [$\mu$l] |
|---|---|---|
| 1 | PCR Grade Nucleotide mix | 2 |
| | R_Primer (VR) | 10 |
| | F_primer (VF2) | 10 |
| | Template DNA | From colony |
| | Sterile water | 28 |
| | Total Volume | 50 |
| 2 | PCR Reaction buffer 10 x | 10 |
| | Taq DNA Polymerase | 2.5 |
| | Sterile water | 37.5 |
| | Total Volume | 50 |

Table 9: PCR condition using Taq polymerase

| Step | Cycles | Temperature {°C} | Time |
|---|---|---|---|
| Initial Denaturation | 1 | 94 | 2 min |
| Denaturation | 30 | 94 | 30 sec |
| Annealing | | 52 | 1 min |
| Extension | | 72 | 1 min |
| Final Extension | 1 | 72 | 7 min |
| Hold | indefinitely | 4 | - |

## Results and Conclusions

The result of the PCR with the verification primers show bands of the wrong size. The amplified fragment should be 941 bp in size, however the gel shows bands shorter than 500 bp which indicated that the wrong insert was cloned into the vector.

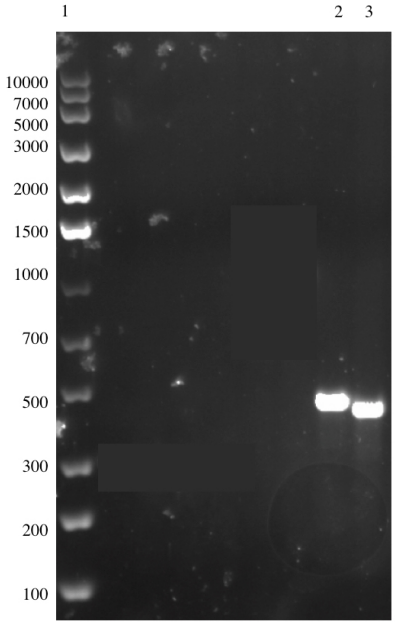


Figure 2: Colony PCR of cells transformed with pSB1C3-Gb1-SortA. (1) Ladder (2) Colony 1 (3) Colony 2

## Discussions and Troubleshoot

It was likely that the contamination from the PCR of Gb1-SortA was digested and ligated into the backbone. The primers used for the amplification of Gb1-SortA was long and had a high GC content. Therefore, unspecific bands could have been amplified during the PCR and as we did not do a gel purification of the PCR product, these contaminating bands could have been ligated cloned instead of the Gb1-sortA fragment.

# 4 PCR of Gb1-SortA

## Responsible

Reskandi Rudjito

## Protocols used

- PCR
- Gel electrophoresis

# Experimental Set Up

The previous ligation of pSB1C3-Gb1-SortA had shown that the wrong fragment was ligated into the backbone. Therefore this experiment serves to amplify more Gb1-SortA containing the correct suffix that would be used for re-ligation.
The primers used in this PCR were:
- Prefix F

5'CACGAATTCGCGGCCGCTTCTAG'3

- GS1 Suffix R

5'AAACTGCAGCGGCCGCTACTAGTATTAGTGGTGATGGTGATGATGTTTGACTTCTGTAG'3

Table 10: PCR Reaction of Gb1-SortA

| Master Mix | Component | Volume [$\mu$l] |
|---|---|---|
| 1 | PCR Grade Nucleotide mix | 1 |
| | R_Primer (VR) | 5 |
| | F_primer (VF2) | 5 |
| | Template DNA | 2 |
| | Sterile water | 14 |
| | Total Volume | 25 |
| 2 | PCR Reaction buffer 10 x | 5 |
| | Taq DNA Polymerase | 0.5 |
| | Sterile water | 19.5 |
| | Total Volume | 25 |

Table 11: PCR condition using Taq polymerase

| Step | Cycles | Temperature {°C} | Time |
|---|---|---|---|
| Initial Denaturation | 1 | 94 | 2 min |
| Denaturation | 30 | 94 | 30 secs |
| Annealing | | 66 | 1 min |
| Extension | | 72 | 1 min |
| Final Extension | 1 | 72 | 7 min |
| Hold | indefinitely | 4 | - |

## Results and Conclusions

The amplified product should be 677 bp in size. This is slightly longer than the previous band of 662 bp where the suffix was incorrectly designed in the primer. The bands on the gel below show bands of fragments between 500-700 bp. The bands were subjected to gel purification to remove unspecific bands at around 200-300 bp. Gel purification was done on the strong band close to the 500 bp ladder and on the smear next to the 700 bp ladder.

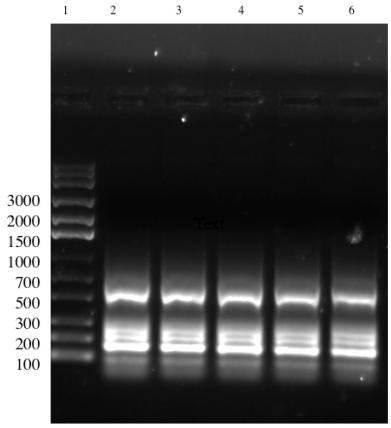


Figure 3: PCR of Gb1-SortA. (1) Ladder (2)-(6) Gb1-SortA

Table 12: Gel purification of Gb1-SortA

| Component | Concentration [ng/$\mu$l] |
|---|---|
| Gb1-SortA (Strong band) | 21.09 |
| Gb1-SortA (Smear) | 7.08 |

# 5 Digestion Gb1-SortA and Ligation of pSB1C3-Gb1-SortA

## Responsible

Aman Mebrahtu

## Protocols used

- Digestion and Ligation

## Modifications and comments to protocols

The ligation reaction was not subjected to heat kill.

# Experimental Set Up

The experimental set up for the digestion reaction are summarized below.

Table 13: Concentrations of DNA to be digested

| Sample | Concentration [ng/$\mu$l ] |
|---|---|
| pSB1C3 | 41.0 |
| Gb1-SortaA | 3.6 |

Table 14: Digestion reaction of pSB1C3

| Reaction | Volume [$\mu$l ] |
|---|---|
| DNA | 5 |
| Buffer SH 10 X | 1.0 |
| Pst1 | 0.2 |
| EcoR1 | 0.2 |
| Sterile water | 3.6 |

Table 15: Digestion reaction of Gb1-SortA

| Reaction | Volume [$\mu$l ] |
|---|---|
| DNA | 15 |
| Buffer SH 10 X | 2 |
| Pst1 | 1 |
| EcoR1 | 1 |
| Sterile water | 1 |

The digested products were then purified and measured with nanodrop. Once the concentration was known, the fragments were ligated with T4 DNA Ligase.

Table 16: Concentration of Digestied Products

| Reaction | Concentration [ng/$\mu$l ] |
|---|---|
| Gb1-SortA | 2.4 |
| pSB1C3 | 6.0 |

The insert:vector molar ratio used in the ligation was 5:1. The calculations shown on the tables below are adjusted so that each reaction contains 50 ng of backbone and the inserts are adjusted according to the desired molar ratio. The final reaction volume was 25 $\mu$l.

Table 17: Ligation Reaction of pSB1C3-Gb1-SortA

| Component | Volume [$\mu$l] |
|---|---|
| Gb1-SortA | 15.84 |
| pSB1C3 | 4.17 |
| T4 DNA Ligase | 0.5 |
| T4 DNA Ligase Buffer 10X | 2.5 |
| Sterile water | 1.99 |

## Results and Conclusions

The ligation product was analyzed with gel electrophoresis to see if ligation had worked.

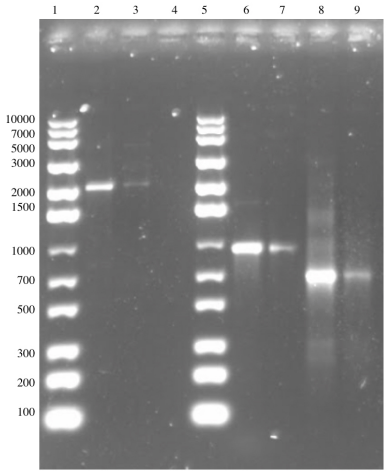


Figure 4: Ligation of pSB1C3-Gb1-SortA. (1) Ladder (2) Digested pSB1C3 (3) Ligation of product of pSB1C3-Gb1-SortA (4) Not of interest (5) Ladder (6) -(7) Not of interest (8) Digested Gb1-SortA (9) Digested Gb1-SortA

## Discussions and Troubleshoot

Based on the gel (Figure 4) we see that there is a faint band around the same size as the backbone (2200 bp) on Lane 3. This lane represents the ligation product. We also see that the digested Gb1-SortA (668 bp) are present in the sample, this is shown by lane 8 and 9. The presence of these bands is a good foundation to continue with transformation. Transformation was later performed in TOP 10 and BL21 cells, however, the transformation had been unsuccessful.

# 6 Digestion of more Gb1-SortA PCR Product

## Responsible

Reskandi Rudjito and Betty Tesfai

## Protocols used

- Digestion and Ligation

## Modifications and comments to protocols

The amount of enzyme used was adjusted to the concentration of the DNA sample.

# Experimental Set Up

Table 18: Concentrations of DNA to be digested

| Sample | Concentration [ng/$\mu$l ] |
|---|---|
| Gb1-SortaA | 21.9 |

Table 19: Digestion reaction of Gb1-SortaA

| Reaction | Volume [$\mu$l ] |
|---|---|
| DNA | 10 |
| Buffer SH 10 X | 2 |
| Pst1 | 0.2 |
| EcoR1 | 0.2 |
| Sterile water | 7.6 |

# Results and Conclusions

The result of the digestion was subjected to gel electrophoresis. Undigested Gb1-SortA has a size of 677 bp whereas the digested product should is 669 bp in size. The difference in size is unfortunately insignificant on the gel.

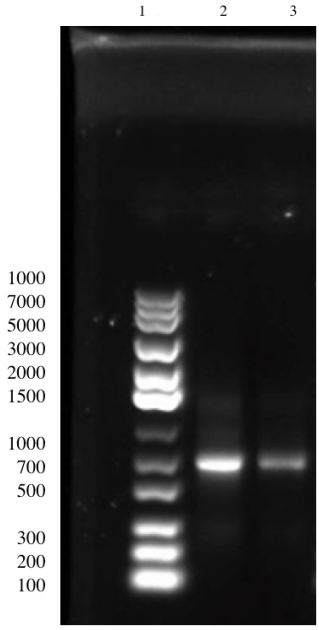


Figure 5: Digestion of Gb1-SortA. (1) Ladder (2) Undigested Gb1-SortA (3) Digested Gb1-SortA